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TITLE: The Effects of Fatty Acids on Retinoid Signaling in Human
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13. ABSTRACT (Maximum 200 Words) Retinoic acid (RA) regulates the proliferation of a wide variety of cell types through the action of retinoic acid receptors. Phytanic acid (PA) and docosahexaenoic acid (DHA) are diet-derived fatty acids that bind to retinoid X receptors (RXR). Therefore, we hypothesized that inhibitory effects on cell proliferation may be enhanced by the addition of PA and DHA to RA-treated cells. We demonstrate that 1) the combination of PA or DHA with RA resulted in enhanced growth arrest of estrogen receptor positive human breast cancer (HBC) cells; 2) PA and DHA induced growth arrest of estrogen receptor negative HBC cells; 3) synthetic RXR agonists induced growth inhibitory effects similar to PA and DHA in HBC cells; and 4) PA enhanced RA-induced expression of CYP26 mRNA in HBC cells and in murine embryonic stem cells. Our data indicate that PA and DHA may be useful adjuvant agents when retinoids are used to inhibit cell proliferation and/or to induce cell differentiation. Deciphering the effects of diet-derived RXR agonists may lead to new therapeutic and experimental uses of these agents in combination with retinoids.				
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Introduction

Retinoids are the natural and synthetic derivatives of vitamin A (retinol), and function as steroid-hormone-like molecules in regulating diverse biological processes such as cellular proliferation, differentiation, and apoptosis (1). Within target tissues, retinoid actions are mediated by the retinoic acid receptors (RARs) and retinoid X receptors (RXRs) (2, 3). RARs and RXRs are ligand-activated transcription factors that bind as RXR:RAR heterodimers to retinoic acid response elements (RAREs), specific DNA sequences in target gene promoters (2, 3). Ligand binding causes the dissociation of co-repressor proteins and promotes association of co-activators, resulting in activation of gene transcription (2, 3). Although a growing number of genes has been identified as retinoid-responsive, the mechanisms by which these gene products regulate biological responses are not fully delineated.

Retinoid signaling pathways are involved in the inhibition of cancer development and progression, and retinoids are currently used pharmacologically as chemopreventive agents against skin, head and neck, breast, liver, and other forms of cancer (4, 5). Retinoids are promising chemopreventive agents for human breast cancer (HBC) (4, 5). Numerous studies have shown that RA inhibits the proliferation of some, but not all, HBC cell lines (6-13). Most retinoid-induced growth inhibition is limited to estrogen receptor (ER)-positive HBC cell lines, while most ER-negative HBC cell lines are resistant to the growth inhibitory effects of retinoids (14).

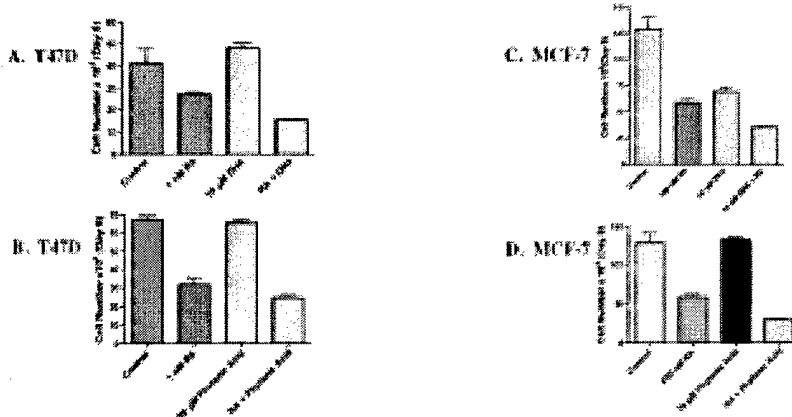
Combination chemopreventive strategies for breast cancer are being studied in order to enhance intrinsic physiological mechanisms that prevent the development and progression of cancer. The fatty acids docosahexaenoic acid (DHA) and phytanic acid (PA) are agonists for RXRs (15-18). DHA, an omega-3 fatty acid found in fatty fish, inhibits proliferation of breast cancer cells in both animal models and in cell culture studies (19, 20). Since RA is an agonist of RARs, DHA and PA are agonists of RXRs, and RXR:RAR heterodimers function to mediate retinoid target gene transcription, we hypothesized that these diet-derived compounds might be combined with RA in breast cancer chemoprevention. In this study, we evaluated the effects of RAR- and diet-derived RXR-selective agonists on the growth of RA-sensitive and RA-resistant HBC cells.

Body

DHA and PA in combination with RA enhance growth arrest in RA-sensitive MCF-7 and T47D HBC cells.

I used growth curve assays to study the proliferation effects of the RA-sensitive HBC cell lines, MCF-7 and T47D. RA inhibits the proliferation of RA-sensitive T47D and MCF-7 HBC cells, whereas it has almost no inhibitory effect on RA-resistant MDA-MB-468 HBC cells (6, 7). At 1 nM, RA inhibited the growth of T47D cells by $34\% \pm 1.8\%$ (Figure 1A). DHA alone ($10\text{ }\mu\text{M}$) stimulated the growth of T47D cells by $18\% \pm 4.5\%$ (Figure 1A). In contrast, when T47D cells were treated for 6 days with a combination of 1 nM RA and $10\text{ }\mu\text{M}$ DHA, the growth of these cells was inhibited by $62\% \pm 1.1\%$ as compared to the control (Figure 1A). PA alone ($20\text{ }\mu\text{M}$) inhibited the proliferation of T47D cells by $3\% \pm 2\%$ (Figure 1B). The combination of 1 nM RA and $20\text{ }\mu\text{M}$ PA inhibited the proliferation of T47D cells by $63\% \pm 2.0\%$ (Figure 1B). RA alone (100 nM), and $10\text{ }\mu\text{M}$ DHA alone inhibited the proliferation of MCF-7 cells by $54\% \pm 2.3\%$ and $47\% \pm 3.7\%$, respectively (Figure 1C). The combination of RA and DHA inhibited the proliferation of MCF-7 cells by $72\% \pm 0.4\%$ after 8 days (Figure 1C). The combination of 100 nM RA and $10\text{ }\mu\text{M}$ PA inhibited the proliferation of MCF-7 cells by $77\% \pm 1.1\%$ after 8 days, compared to a $54\% \pm 2.3\%$ inhibition with 100 nM RA alone (Figure 1D).

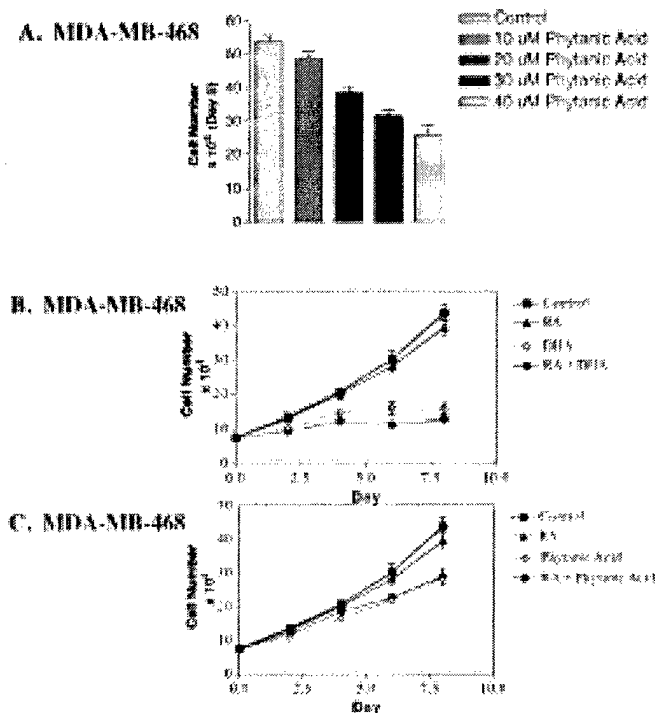
Figure 1.



DHA and PA inhibit the proliferation of RA-resistant MDA-MB-468 HBC cells.

PA inhibited the proliferation of MDA-MB-468 cells in a dose-dependent manner (Figure 2A). MDA-MB-468 cells were treated for 8 days with 10 μ M DHA or 20 μ M PA alone, or in combination with 1 μ M RA. RA inhibited MDA-MB-468 proliferation by only 10% at 8 days (Figure 2B and 2C). In contrast, DHA and PA were more effective at inhibiting the proliferation of these cells. Treatment with 10 μ M DHA led to a 64% \pm 3.7% growth inhibition at 8 days (Figure 2B), and treatment with 20 μ M PA led to a 36% \pm 3.9% growth inhibition at 8 days (Figure 2C). Treatment with DHA and PA in combination with 1 μ M RA did not enhance growth inhibition as compared to DHA or PA alone (Figure 2B and 2C), in contrast to what we observed in MCF-7 and T47D cells (Figure 1).

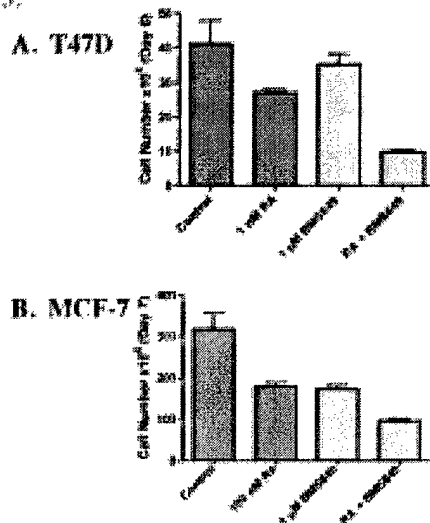
Figure 2.



Synthetic RXR agonists enhance the growth inhibitory effects of RA in RA-sensitive T47D and MCF-7 HBC cells.

RARs and RXRs bind as RXR:RAR heterodimers to RAREs (2, 3) and there is a synergistic interaction between RAR and RXR agonists (21). If the growth inhibitory effects of DHA and PA are mediated through RXRs, then synthetic RXR agonists should produce similar growth inhibitory effects in T47D and MCF-7 cells. T47D cells were treated for 6 days with 1 μ M of the RXR-agonist BMS649 alone, or in combination with 1 nM RA. Treatment with RA alone or the RXR agonist BMS649 alone resulted in growth inhibition of $34\% \pm 1.8\%$ and $15\% \pm 7.4\%$, respectively (Figure 3A). Treatment with RA and the RXR-agonist BMS649 in combination led to a $76\% \pm 1.5\%$ growth inhibition (Figure 3A). In MCF-7 cells, the combination of RA and the RXR agonist BMS649 led to a $70\% \pm 1.0\%$ growth inhibition, compared to a $43\% \pm 2.3\%$ and $45\% \pm 2.9\%$ growth inhibition with RA alone or the RXR agonist BMS649 alone, respectively (Figure 3B).

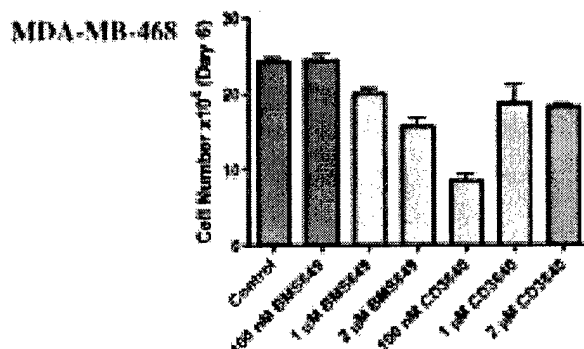
Figure 3.



Synthetic RXR agonists inhibit proliferation of RA-resistant MDA-MB-468 HBC cells.

MDA-MB-468 cells were treated for 6 days with 1 μ M concentrations of the synthetic RXR agonists BMS649 and CD3640. BMS649 inhibited the proliferation of MDA-MB-468 cells by $17\% \pm 2.0\%$ and CD3640 inhibited the proliferation by $23\% \pm 9.7\%$ (Figure 4).

Figure 4.

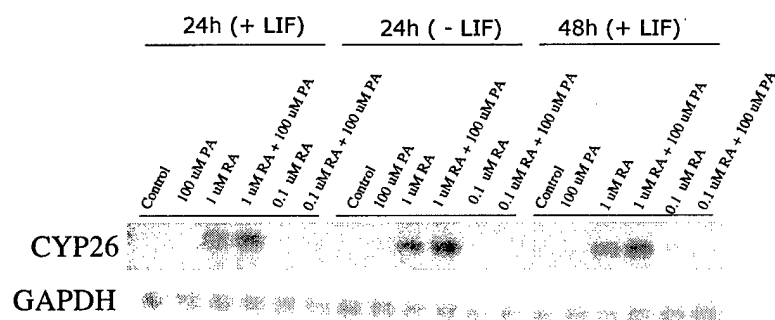


Effects of RA and PA on CYP26 mRNA expression in AB-1 ES cells and MCF-7 cells.

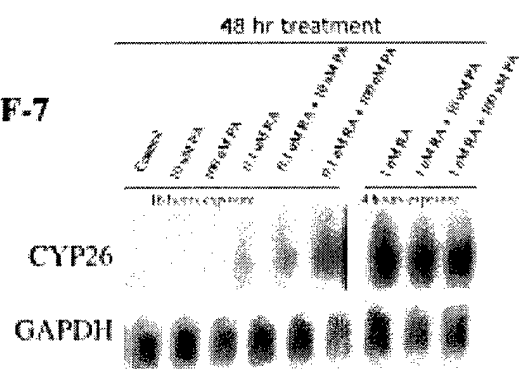
CYP26, also known as P450RAI, is an enzyme which metabolizes RA to more polar metabolites (22). RA induces the expression of CYP26 mRNA (22, 23), and both PA and DHA enhance the induction of CYP26 in RA-treated intestinal cells (24). We measured the level of CYP26 mRNA in the presence and absence of RA and PA. In our preliminary data, we showed that in murine embryonic stem cells, RA induced the CYP26 message, while PA alone did not (Figure 5A). RA induces CYP26 mRNA expression in T47D and MCF-7 HBC cells (25). The addition of PA to RA-treated MCF-7 cells resulted in a small increase in the level of CYP26 mRNA (Figure 5B and 5C).

Figure 5

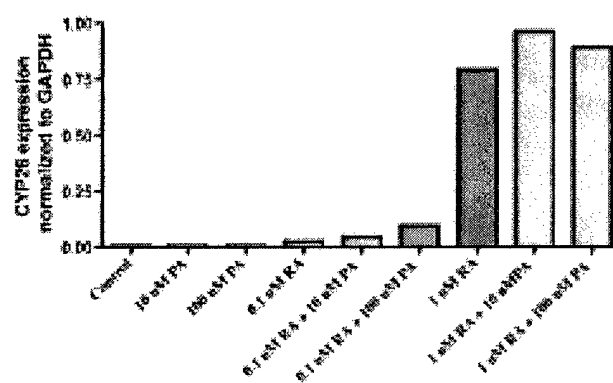
A. AB1 Embryonic Stem Cells



B. MCF-7



C. MCF-7



Research Accomplishments

Evaluated the effects of DHA, PA, RA, and synthetic retinoids on the proliferation of RA-sensitive (MCF-7 and T47D) and RA-resistant (MDA-MB-468) human breast cancer cell lines.

Demonstrated by Northern analysis that CYP26 is induced by RA and this RA-induction is enhanced by the addition of PA in MCF-7 cells.

Completed a manuscript for publication based on these research results

Reportable Outcomes

Tighe, A.P., Langton, S., and Gudas, L.J. Docosahexaenoic Acid, Phytanic Acid, and Retinoic Acid Cause Growth Inhibition of Human Breast Cancer Cells and Murine Embryonic Stem Cells. (2004) Manuscript.

Conclusions

We have shown that the dietary fatty acids, DHA and PA, in combination with RA enhance the growth inhibitory in HBC cells. Our results indicate that it may be possible to lower the dose of clinically used retinoids and attenuate their side effects without compromising their growth inhibitory actions by combining them with diet-derived RXR agonists. Thus, the combination of retinoids with DHA and PA may represent a new chemopreventive strategy for breast cancer.

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